DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

INVESTIGATION OF EFFECTS OF DIFFERENT ENVIRONMENTAL STRESS CONDITIONS ON SOME CYANOBACTERIUM METABOLITES

by

Yusuf KERİMOĞLU

January, 2014

İZMİR

INVESTIGATION OF EFFECTS OF DIFFERENT ENVIRONMENTAL STRESS CONDITIONS ON SOME CYANOBACTERIUM METABOLITES

A Thesis Submitted to the
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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"INVESTIGATION OF EFFECTS OF DIFFERENT ENVIRONMENTAL STRESS CONDITIONS ON SOME CYANOBACTERIUM METABOLITES"** completed by **YUSUF KERİMOĞLU** under supervision of **Prof. Dr. RAZİYE ÖZTÜRK ÜREK** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Yusuf KERİMOĞLU

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ABSTRACT

Cyanobacteria are the most elementary shapes of life on earth, and their simple prokaryote cells are carrying out photosynthesis. They can grow in a wide habitat and survive at different conditions. *Spirulina platensis* is one of the most known cyanobacterium which has a great biomass production as well as potential source of many metabolites.

The aim of the study was to investigate the effect of magnesium (0-4.057 mM), copper (0-5 μM) and temperature (37 degrees Celsius) on the biomass and levels of protein, Chl *a*, phycobiliproteins, carotenoids, MDA, proline, and metal contents of *S. platensis*, as well as PSII activity.

The highest biomass was determined on control conditions including both magnesium (0.8 mM) and copper (0.5 μM) as 49.1 mg/mL. Protein level was also higher at 0.5 μM copper throughout incubation period. Although CPC, APC and Chl *a* levels were varied by changing concentrations of magnesium, CPC and APC were major fraction and also higher Chl *a* levels observed at control copper concentration during incubation. On 12^{th} day, increasing copper concentrations caused to decrease on carotenoid level while rising magnesium concentrations supported to production of it. The major carotenoids fraction was β-carotene for studied concentrations both metals as being highest value, 2396.73 μg/mL for inexistence of copper. However, lower or upper control conditions of both metals were caused higher MDA content. Proline accumulation was detected as higher for initial days during cultivation period for any condition. Also, PSII activities were decreased by increasing of temperature from 30 to 37 degrees Celsius. In addition, different magnesium and copper metal concentrations (excessive and insufficient) were caused to decrease on PSII activities. Apart from these, metal interactions were evaluated in control condition,

excess or absent of magnesium and copper. Different metal contents of *S. platensis* were expressed depending on magnesium and copper concentrations.

Keywords: Carotenoids, phycobiliproteins, chl *a*, *S. platensis*, magnesium, copper, temperature, PSII activity.

FARKLI ÇEVRESEL STRES KOŞULLARININ BAZI SİYANOBAKTERİ METABOLİTLERİ ÜZERİNE ETKİLERİNİN ARAŞTIRILMASI

ÖZ

Siyanobakteriler yeryüzündeki yaşamın en temel formlarıdır ve basit prokaryotik hücreleriyle fotosentez yapabilirler. Siyanobakteriler geniş bir habitatta gelişebilir ve farklı koşullarda yaşayabilirler. *Spirulina platensis*, büyük biyokütle üretimi yanında birçok metabolitin potansiyel kaynağına sahip en çok bilinen siyanobakterilerden biridir.

Çalışmanın amacı, magnezyum (0-4.057 mM), bakır (0-5 µM) ve sıcaklığın (37 santigrat derece) *S. platensis*'in biyokütle, klorofil *a*, fikobiliproteinler, karotenoidler, MDA, prolin ve metal içeriğinin yanı sıra PSII aktivitesine etkisini incelemektir.

En yüksek biyokütle, magnezyum (0.8 mM) ve bakır (0.5 µM) içeren kontrol koşullarında 49.1 mg/mL olarak saptandı. Protein düzeyi de 0.5 µM bakır da inkubasyon periyodu boyunca daha yüksekti. CPC, APC ve klorofil *a* düzeyleri farklanan magnezyum konsantrasyonlarıyla değişmesine karşın, kontrol bakır konsantrasyonunda inkübasyon süresince CPC ve APC temel fraksiyondu ve de daha yüksek klorofil *a* düzeyleri elde edildi. 12. günde, artan magnezyum derişimleri karotenoid üretimini desteklerken, artan bakır konsantrasyonları karotenoid düzeyinde azalışa neden oldu. Bakır olmayan koşulda en yüksek değer olarak 2396,73 µg/mL olan β-karoten iki metalin çalışılan bütün konsantrasyonlarında temel karotenoid fraksiyonuydu. Bununla birlikte, kontrol koşullarının üstünde veya altındaki derişimler iki metal için de daha yüksek MDA içeriğine neden oldu. Bütün koşullarda, kültivasyon peryodunun ilk günlerinde daha yüksek prolin artışı saptandı. Aynı zamanda, PSII aktivitesi, 30 santigrat dereceden 37 santigrat dereceye arttırılan sıcaklık ile azaldı. İlaveten, farklı magnezyum ve bakır metal derişimleri (aşırı ve yetersiz), PS II aktiviteleri üzerinde azalışa neden oldu. Ayrıca, metal etkileşimleri, magnezyum ve bakırın aşırı, kontrol ve yokluk koşullarında değerlendirildi. *S.*

platensis'in farklı metal içerikleri, magnezyum ve bakır derişimlerine bağımlı olarak ifade edildi.

Anahtar kelimeler: Karotenoidler, fikobiliproteinler, klorofil *a*, *S. platensis*, magnezyum, bakır, sıcaklık, PSII aktivitesi.

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CHAPTER ONE
INTRODUCTION

In the different aquatic ecosystems and terrestrial habitats such as rocks, caves, soils, buildings, and living beings and plants as well as extreme environments like deserts, polar regions, hotsprings or toxic sulfurous or metal rich environments around 10 million species of cyanobacteria and eukaryotic algae on the world accommodate (Hoffmann, 1989; Adams, 2000; Guiry & Guiry, 2012). Although they are known to exist on nature like freshwater, estruaries, and oceanic environments, the existence of algae at the same time everywhere and evidence of their presence in numerous stromatolites and the Mesozoic period support to the belief of they are the preliminary organisms to seen on the earth's surface (Riding, 2011). However they are among the most elementary shapes of life on earth, their simple prokaryote cells are carrying out photosynthesis like plants but having no plant cell walls such as primitive bacteria (Singh & Kate, 2005).

Algal phylogeny encompasses ten major phyla (prokaryotic Cyanophyta and Prochlorophyta, and the eukaryotic Glaucophyta, Euglenophyta, Cryptophyta, Haptophyta, Dinophyta, Heterocontophyta (including diatoms, brown algae), Rhodophyta (Red algae), and Chlorophyta (green algae)) (Mur et al., 1999). Cyanobacteria are member of the kingdom Monera and division Cyanophyta. They are wider than other bacteria and commonly hydrophilous. They are named as it often known ‘blue- green algae’ due to being photosynthetic and hydrophilous (Singh & Kate, 2005). In addition Cyanobacteria, taxonomically grouped under the Gram-negative prokaryotes by microbiologists, and as a Division in Plant Kingdom by botanists (Stanier et al., 1971), their sizes and forms are variable from unicells of 1-2 μm in diameter to filaments of even 10 cm.

Cyanobacterial genera are included edible (such as *Nostoc, Spirulina* and *Aphanizpomenon)* and toxic species (*Microcyctis*) (Singh & Kate, 2005). Cyanobacteria have attraction as being a crude unprocessed food which is abundant in phycocyanin, carotenoids, chlorophyll, indispensable amino acids and fatty acids,

other bioactive compounds. The nutrient content is affected by the sitution, environment and locality where the algae are thrived. To be rich in terms of biologically active secondary metabolites is the most prominent property of prokaryotic photosynthetic microorganisms. Inexistence of nuclear membranes, internal organelles and histone proteins associated with chromosomes are announced for truly prokaryotic. They are convenient for utilising carbon dioxide and the reductive pentose phosphate pathway or Calvin cycle is driven by them (Stal & Moezelaar, 1977). While cyanobacteria are carried out oxygenic photosynthesis and contributed to oxygen balance of atmosphere and ocean, all other photosynthetic bacteria perform anoxygenic photosynthesis (Jorgensen, 2001). In addition, some of cyanobacterial species are also suitable for nitrogen fixation and promote the nitrogen balance of soils (Subashchandrabose et al., 2013).

All cyanobacteria are unicellular, though many grow in colonies or filaments, often surrounded by a gelatinous or mucilaginous sheats, depending upon environmental conditions (Singh & Kate, 2005). *Spirulina,* a kind of blue-green algae, thrives naturally spread areas throughout earth and also lakes in Africa, India, China, and South America. For centuries *Spirulina* has been used as food by the natives living near Lake Chad in Central Africa and near Lake Mexico (Borowizka, 1988). Wild *Spirulina* was also used as a food by Aztecs.

Spirulina platensis is a planktonic photosynthetic filamentous cyanobacterium that can grow at alkaline pH values of up to 11 in tropical and subtropical bodies of water which is balanced by carbonate, bicarbonate and inorganic nitrogen and forms massive populations (Aiba & Ogawa, 1977; Colla et al., 2007). The main morphological features of genus, i.e. the arrangement of multicellular cylindrical trichomes in an open left-hand helix along the entire length of filaments, help us to recognize this cyanobacterium (Vonshak, 1997). Due to lack a nucleus surrounded by a membrane and the absence of the differentiated cell structures *Spirulina* is grouped as a prokaryotic microorganism and their cellular structure is more complicated than the others (Russ Mason, 2001). *S. platensis* has been largely studied due to its commercial importance as a source of protein, vitamins, essential

amino acids, and fatty acids (Aiba & Ogawa, 1977; Umesh & Sheshagiri, 1984). *Spirulina* has 62-percent amino acid content, mixed carotenoids and xanthophyll phytopigments, a high concentration of vitamin B_{12}, immunoregulatory polysaccharide components such as β-glucan, and abundant amounts of gamma linolenic acid, which plays a critical role in inflammatory processes (Russ Mason, 2001).

1.1 Photosynthesis

Photosynthesis is one of the most important biochemical processes on the Earth by which phototrophic organisms such as plants, algae and cyanobacteria harvest light energy from the abundant flux of sunlight incident on the Earth and convert it into chemical energy (Figure 1.1). In this complex process, the solar energy which is captured by pigments (chlorophyll, phycobiliprotein, carotenoids) is utilized to combine water, carbon dioxide, and minerals into oxygen and storage organic compounds which are rich in chemical energy and required to maintenance forms of life.

In the equation, six molecules of water, six molecules of carbon dioxide and light energy are combined and produced one molecule of sugar and six molecules of oxygen. The overall reaction can be written as:

$$6H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2$$

Photosynthesis is a mechanism in which is driven a number of photochemical and enzymatic reactions. It is separated in two distinct stages, the light reactions (oxygenic photosynthesis), which convert light energy to ATP and NADPH (nicotinamide adenine dinucleotide phosphate); and the dark reactions, which convert CO_2 to carbohydrate using ATP and NADPH.

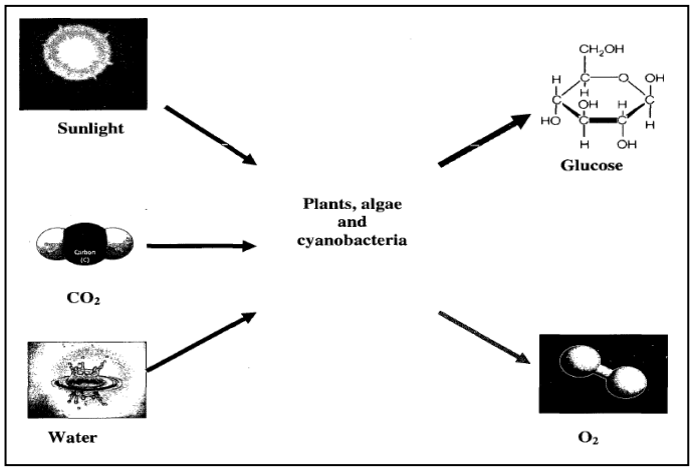

Figure 3.1 Photosynthesis: Plants, algae and cyanobacteria use the energy from sunlight and convert CO_2 and water into organic compounds and oxygen (Chauhan, 2009).

During the light-dependent stage, chlorophyll and carotenoids function as absorbing light energy, which excites the pigment molecules to higher energy level. The energy is transferred from the antenna pigments to the reaction center cores of Photosystem I (PSI) and Photosystem II (PSII), where charge separation takes place. The electrons for this process are provided by PSII by oxidation of water to O_2 and protons. At the end of the light reactions, high-energy molecules NADPH and ATP are consisted and O_2 is released as a by-product.

$$12H_2O + 12NADP + 18ADP \rightarrow 6O_2 + 12NADPH + 18ATP$$

Products of light reactions (NADPH and ATP) spend in the second stage, the dark reaction (Calvin cycle) which is independent to light and does not require it. In this stage, glucose is generated by using atmospheric carbon dioxide, simultaneously.

$$6CO_2 + 12NADPH + 18ATP \rightarrow C_6H_{12}O_6 + 12NADP + 18ADP + 6H_2O$$

The reactions of generated biochemical energy and molecular oxygen as a byproduct are occurred within the thylakoid membranes, in which a transmembrane charge separation is catalyzed by PSI and PSII which are multisubunit membrane protein complexes of the photosynthetic electron transfer chain as other complexes are the cytochrome b_6f complex, ATP synthase.

1.2 Photosynthetic Apparatus of *S. platensis*

1.2.1 PS II

PSII is a large homodimeric multisubunit membrane protein complex which functions as molecular machine and that is responsible for carrying out light-induced electron flux and water-splitting reactions, leading to the released molecular oxygen and removing protons into thylakoid lumen (Loll et al., 2005; Fromme et al., 2001; Grotjohann & Fromme, 2005; Guskov et al., 2009). P680 is primary electron donor of PSII. When P680 excited, charge separation takes place. Plastoquinone (PQ) is the final electron acceptor in PSII. Depending on double reduction, it takes up two H^+ at the stromal side, migrates in the lipid bilayer, and transfers the electrons to another membrane bound protein complex, the cytochrome (Cyt) b_6f complex. The (Cyt) b_6f complex removes electrons from the reduced, membrane-soluble plastoquinol molecule and transfers them to the small soluble electron carrier plastocyanin (PC), copper protein in the thylakoid lumen (Fromme & Mathis, 2004; Roose, 2008). Instead of PC, another electron carrier can exist, as being Cyt c_6 in some cyanobacteria.

1.2.2 PSI

PSI is the largest and most complex membrane protein which is a heterodimer and contains 11 protein subunits, 96 Chl *a* molecules, 22 carotene molecules, 3 Fe_4-S_4 clusters and 2 phylloquinones (Chitnis, 2001; Fromme et al., 2001). Electron donor (P700) and electron acceptors (A_0, A_1), and Fe_4-S_4 clusters are ligated to these subunits. P700 has a dimeric structure which consists of two molecule Chl *a* while the primary electron acceptor A_0 is a monomeric form of Chl *a*, and A_1 is a phylloquinone, functions A_0 between an iron-sulfur center Fx (iron-sulfur cluster of PSI) of the [4Fe-4S]. Within this large antenna structure as absorbing spread spectrum light energy, PSI catalyzes the second step of the electron transfer chain by transferring electrons from PC to ferredoxin (Fd) and NADPH which consist by reducing $NADP^+$ on the stromal/cytoplasmic side of the membrane, generated by

ferredoxin NAPD$^+$ reductase (FNR). While electron transfer reactions are occurred, protons flux from the stromal to the lumenal side of the membrane as driven gradient power of ATP synthesis by ATP synthase, simultaneously. The redox diagram from P680 to P700 resemble to Z so that the electron transfer pattern is known the Z scheme of photosynthesis (Fig 1.2a, b).

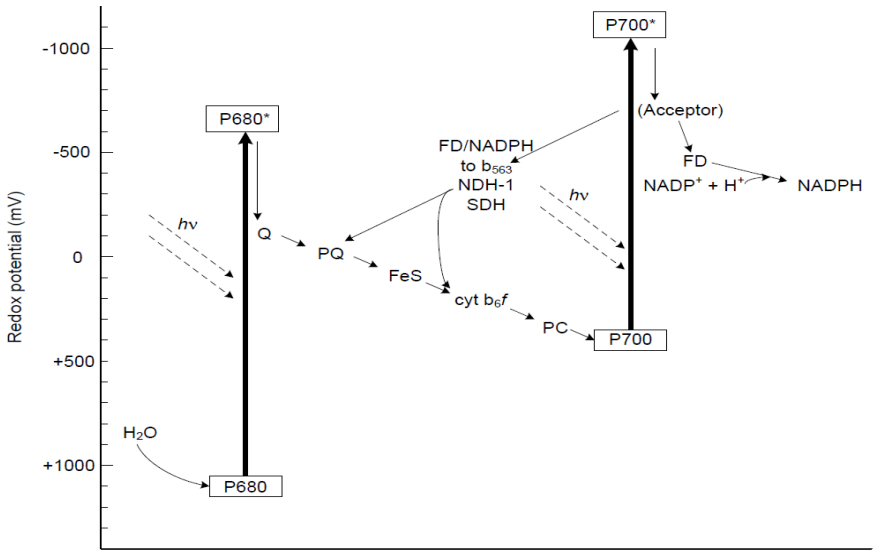

Figure 1.2.a "Z-scheme" of excitation energy transfer through the electron transport chain in *S. platensis*. Excitation is provided by the photosystems, beginning with the oxidation of water and concluding with the reduction of NADP$^+$.

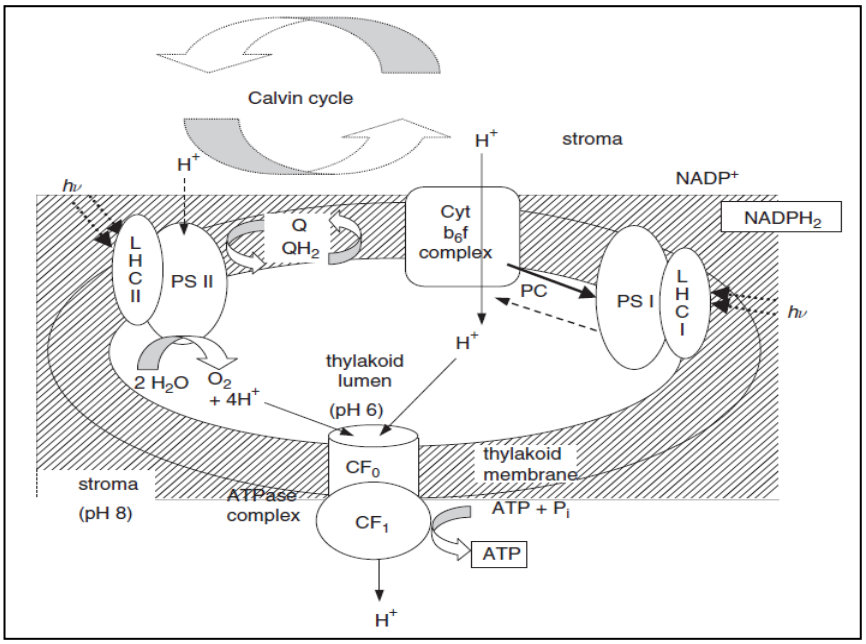

Figure 1.2.b Vectorial arrangement of photosystem I and II, the cytochrome b6/f complex, and the ATP synthase within the thylakoid (adapted from Stryer, 1988).

The ATP and NADPH produced by the light reactions are used in the light independent reactions for the synthesis of organic compounds from CO_2. In the absence of $NADP^+$ or under severe ATP demand, cells fallow a different mechanism known cyclic-photophosphorylation. This process is not driven on the PSII and it produces neither NADPH nor O_2. Contrary, electron transfer reactions in PSI drive a cyclic electron flow in which the electrons are shuttled between PSI and the Cyt b_6f, complex. This cyclic electron flow around PSI produces an electrochemical H^+ gradient across the membrane, which uses to power ATP synthesis (Fork & Herbert, 1993; Bendall & Manasse, 1995; Govindjee & Krogmann, 2004).

1.3 Chlorophyll

Incident light from the sun onto the surface of the Earth is important as it can be absorbed due to being source and sustainability of life. Many organic molecules which are conjugated aromatic structures have ability to absorb light efficiently. Although many beneficial biological compounds that absorb light present, the chlorophylls (Chls) absorb most of the energy productively. The ability of Chls to transfer of energy on the other molecules that is result of productive absorption of light makes them most valued by giving a paramount feature. Chls bind to proteins in light-harvesting complexes, and the core reaction complexes of photosynthesis and the synthesis of high-energy chemical products is carried out by flow of absorbed energy through these complexes (Hoober, 2012).

Chl, a dark green pigment, and main substituent for photosynthesis, is one of the most complex and abundant organic metal compounds with low molecular weight (Takamiya et al., 2000; Tanaka & Tanaka, 2006). Chlorophyll molecules have variety such as Chl *a*, Chl *b*, Chl *c* and Chl *d*. Almost of microorganisms and plants possess Chl *a*, as present the other sort Chls in some.

Photosynthesis for energy production is used by cyanobacteria and high levels of Chls *a* and *b* are accumulated simultaneously. Chls play a centrical role in the light-dependent processes of photosynthesis that are carried out by integral membrane proteins of the thylakoid membranes (Hoober et al., 2007; Lokstein & Grimm, 2007;

Schmid, 2008). Chl converts absorbed light energy from the sun to chemical energy within carbohydrates, which form a part of the cell contents, and utilize as energy by the cyanobacteria (Deshpande, 2005).

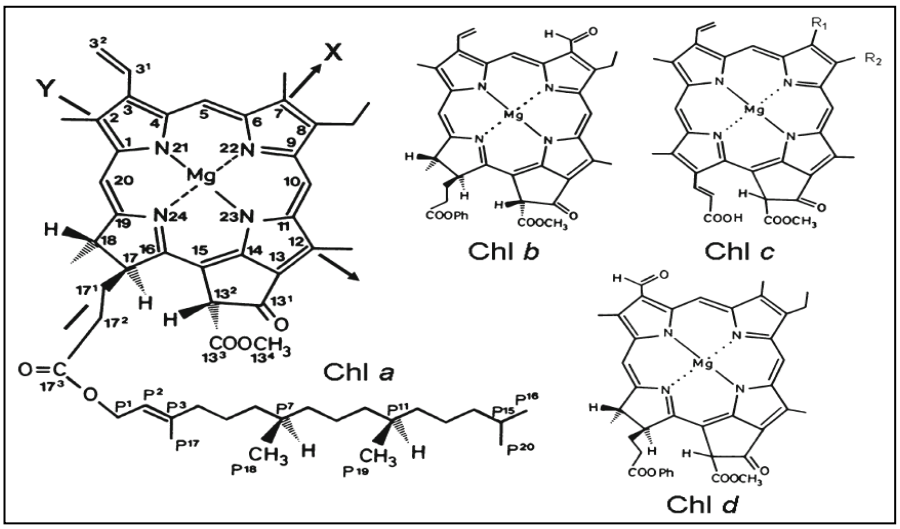

Figure 1.3 Structures of the major chlorophylls. All Chls are tetrapyrroles, where nitrogen atoms are co-ordinated around a Mg atom. Except for the oxidation of the 7-methyl group in Chl *a* to the formyl group in Chl *b*, Chls *a* and b are identical. Chl *d* contains a formyl group at position 3. Chl *c*, also contains double bonds in the side chain between positions 17 ^{1} and 17 ^{2} and in the macrocycle between carbons 17 and 18 (Hoober, 2012).

Chlorophylls are responsible for harvesting solar energy in photosynthetic antenna systems, and for charge separation and electron transport within reaction centers (Tanaka & Tanaka, 2006). Different light sources will generate distinct energy spectra (Carvalho et al., 2006), and this energy spectrum is very important, since only light of specific wavelengths is utilized for photosynthesis. The photosynthetically active radiation is limited to the visible light of the energy spectrum, corresponding to wavelengths in the range of 400 to 700 nm. Since chlorophyll *a* and *b*, as well as a limited number of carotenoid pigments absorb light, only certain regions of the white light spectrum can be trapped by these pigments and subsequently used for photosynthesis. The chlorophyll absorption wavelengths correspond to the blue (475 nm) as well as the red (650 nm) spectral regions (Matthijs et al., 1996). Red light contains energy needed to reach the first excited state of chlorophylls *a* and *b*, while the blue light has more energy and can be

elevated to higher exited state (Matthijis et al., 1996). Carotenoids usually absorb in the 480 to 560 nm range, the part of the energy spectrum where the chlorophylls have weak absorption (Larkum et al., 2003).

Although many sources indicate that chlorophyll content increased with incubation time (Tarn & Wong, 1996), regulation of the levels of chlorophyll and other pigment derivatives is highly important. Existence of these molecules in excess level cause to generation reactive oxygen species (ROS) due to being strong photosensitizers. As is known, ROS cause to growth retardation or cell death. High levels of produced O_2 during photosynthesis will inhibit photosynthesis and reduce productivity so it may be hazardous as leading photo-oxidative damage on chlorophyll. Chlorophyll metabolism, therefore, must control to sustain its important functions and to prevent irreversibly effects of ROS and photo-oxidative damages. Chlorophyll metabolism is a highly coordinated process that is executed via a series of cooperative reactions catalyzed by numerous enzymes (Beale, 1996).

A few metals such as Fe, Mn, Zn and Cu are especially essential for photosynthesis. Iron is quantitatively the most important trace metal involved in photosynthesis, followed by manganese that plays essential role in O_2 evolution (Raven et al., 1999). In the medium, Fe deficiency condition causes to retardation of algal growth, and reduction of photosynthesis. Moreover, It has been reported that the iron deficiency leads to unwanted impact on function of chlorophyll system (Mandalam & Palsson, 1998; Ozturk Urek & Tarhan, 2011), resulting in small cells that are low in chlorophyll content (Meisch et al., 1980). The structural metal, Mg was needed by cells in synthesis of chlorophyll, while the others such as S and N are functional in cellular division (Mandalam & Palsson, 1998).

1.4 Carotenoids

Carotenoids are lipophylic isoprenoid compounds synthesized by all photosynthetic organisms like plants, algae, and cyanobacteria but also by some nonphotosynthetic bacteria and fungi. Synthesized carotenoids by plants and microorganisms are natural pigments which of the over 600 carotenoids found in nature.

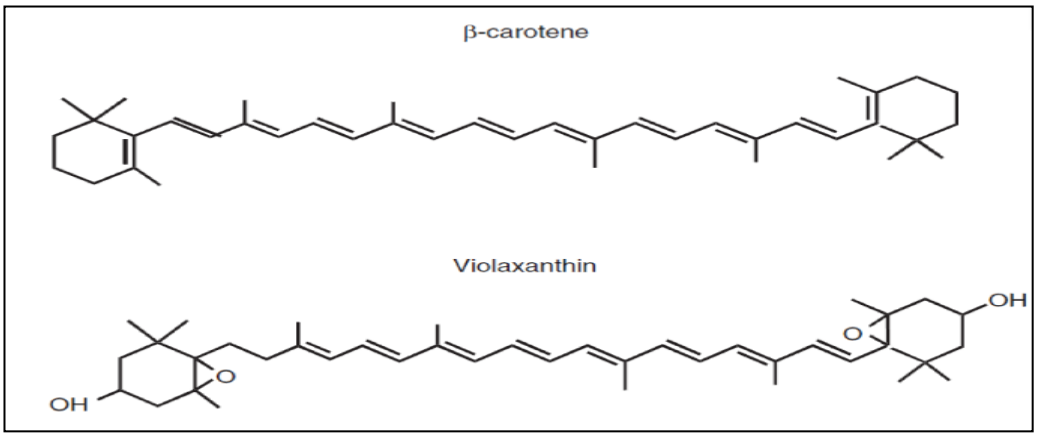

Figure 1.4 The structures of carotenoids. Carotenoids are conjugated isoprenes with cyclic 6-carbon side groups, whereas compared to carotenes, xanthophylls such as violaxanthin, are oxygenated.

Two groups of carotenoids are found in nature. The first group is constituted carotenes like β-carotene; those are the linear hydrocarbons which can be cyclized at one or both ends of molecule. The second group is xanthophylls which are oxygenated derivatives of carotenes such as lutein, violaxanthin, neoxanthin, and zeaxanthin (Botella-Pavia & Rodriguez-Concepcion, 2006). Xanthophylls such as zeaxanthin, lutein, α-and β-cryptoxanthin, canthaxanthin and astaxanthin with hydroxy- and keto- groups as structural elements are important. Almost all carotenoids contain chiral centers, and thus they have stereo-isomeric form varieties. In nature, the all-*trans* form is thermodynamically most stable therefore carotenoids are predominantly present in the all-*trans* configuration. In homogenous solutions, carotenoids have a tendency to isomerize and comprise a mixture of mono- and poly-*cis* isomers along with the all-*trans* form (Stahl et al., 1993). However, processing sources of carotenoids such as fruits and vegetables produces a 10 to 39% increase in *cis*-isomers (Lessin et al., 1997).

In essential, algae have great potential to serve as cell factories for the production of high-value especially ketocarotenoids compounds. However, most unicellular green algae such as *Dunaliella salina* (Ben-Amotz & Avron, 1983) or *Haematococcus pluvialis* (Jin et al., 2006) over-accumulate these secondary carotenoids only when the cells are exposed to abiotic stress, resulting in the concomitant accumulation of storage lipids and a strong reduction or even cessation of growth.

In animals and human, carotenoids especially β-carotene and lycopene, have an important role in the protection against photooxidative processes as an effective scavengers of singlet molecular oxygen and peroxyl radicals and can interact coordinately with other antioxidants (Tapiero et al., 2004). The antioxidant activities of carotenoids are attributed to their singlet oxygen quenching properties and their ability to trap peroxyl radicals (Stahl et al., 1998). The interaction of carotenoids with peroxyl radicals may maintenance by way of an unstable β-carotene radical adduct. Carotenoid adduct radicals are predicted to be relatively nonreactive due to highly resonance stability (Rice-Evans et al., 1997). They may further be exposed to deterioration to generate nonradical products and may terminate radical reactions by binding to the attacking free radicals. Carotenoids are reacted to peroxyl radicals faster than do unsaturated acyl chains by function as antioxidant compounds. This process is resulted in destroying carotenoid molecules (Woodall, et al., 1997). The chemical reactivity of carotenoids toward oxidizing agents and free radicals is related to mainly the polyene chain that is the feature responsible for any antioxidant action. The carotenoids possess quenching activity relating to theirs conjugated double bonds system and end groups. When number of conjugated bonds of molecule present in higher, activity of carotenoids increases. The nature of carotenoid end group is also important parameter on quenching activity of carotenoids. The conjugated double bonds system shows higher influence on quenching activity of carotenoids compared to end groups (cyclic or acyclic) (Britton, 1995). Mixtures of carotenoids are increased effectiveness of any one single carotenoid against lipid peroxidation, and this synergistic effect is most known if lycopene or lutein is present in the mixture (Stahl et al., 1998).

1.5 Phycobiliproteins

Phycobiliproteins are a family of highly soluble and reasonably stable fluorescent proteins derived from cyanobacteria (Saleha et al., 2011). Phycobiliproteins are placed into particles called phycobilisomes which are attached in regular arrays to the external surface of the thylakoid membrane and act as major light harvesting pigments maximizing energy transfer to the chlorophyll-protein complexes in cyanobacteria (Figure 1.5.) (Gantt, 1981). The phycobilisome is placed on the stromal side of PSII, and transmits light increasing energy yield to the internal antenna chlorophylls. The phycobilisome is a multi-protein complex and composed of multiple rods of varying length. Each rod clearly has several stacked disks that are internally themselves hexameric composites of one or more of the phycobiliproteins phycocyanin (CPC), phycoerythrin (PE), phycoerythrocyanin (PEC) or allophycocyanin (APC). In general, chromophore-bearing polypeptides having α and β subunits, which have a molecular weight of around 20 kDa, are consisted of a hemispheric or hemiovoid multi-protein complex, phycobiliproteins (Abalde et al., 1998).

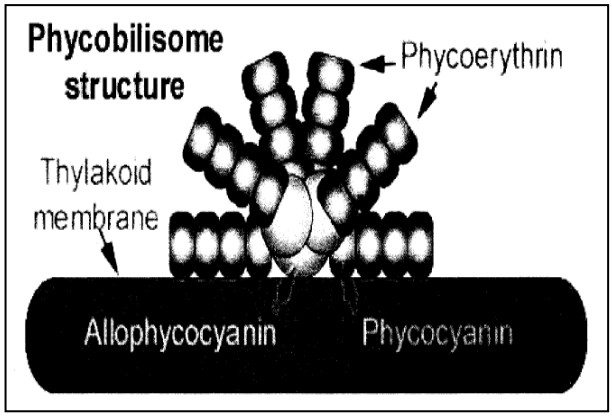

Figure 1.5 A model of a phycobilisome. The rods may be composed of varying proteins, but are always in the forms of 'coin-roll'-like stacks of disks, converging on a trimer of allophycocyanin cylinders (Webb, 2001).

Depending on light conditions and the organism, sort of these proteins may vary it means one or more sort may exist. However, the sequence of energy transfer always

follows the order of Phycoerythrin -> Phycocyanin -> Allophycocyanin -> PSII chlorophyll (Bukhman, 2008).

The phycobiliproteins are proteins with linear tetrapyrrole prosthetic groups, called bilins, found not only in cyanobacteria but also in red algae and cryptomonads (Figure 1.6.) (Bermejo et al., 2002). Phycobiliproteins are divided into three main classes according to their structure: phycoerythrins, phycocyanin and allophycocyanins (Bermejo et al., 2003). Each protein contains a tetrapyrrole chromophore, one of several variations on a covalently-bound bilin (open-chain variant of a porphyrin ring), which is the moiety actually responsible for the protein's color, spectral profiles and its absorbance characteristics.

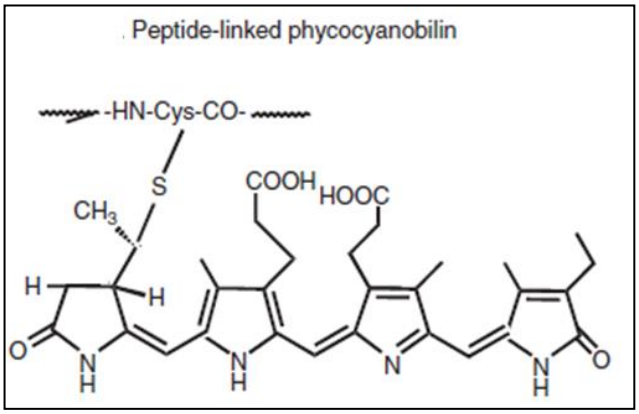

Figure 1.6 Structure of phycobilin. Phycobiliproteins are open tetrapyrroles, which are covalently linked to a protein.

The phycobilisome absorbs light across the 590 - 650 nm range of light wavelengths. As shown in Figure 1.7, this region is a gap in the visible spectrum in which neither chlorophyll nor carotenoids are capable of absorbing light, and the ability of the phycobilisome to effectively utilize energy in these wavelengths conveniently allows the organism greater access to usable light within the visible spectrum and, therefore, greater adaptive ability (Bukhman, 2008). Allophycocyanin, having a λ max of 650–655 nm, occurs as a trimer (α3β3) close to neutral pH conditions, whereas CPC, having a λ max of 610–620 nm, is found as a complex solution of trimers (α3β3), hexamers (α6β6), and other oligomers (MacColl, 1998; Silva et al., 2009).

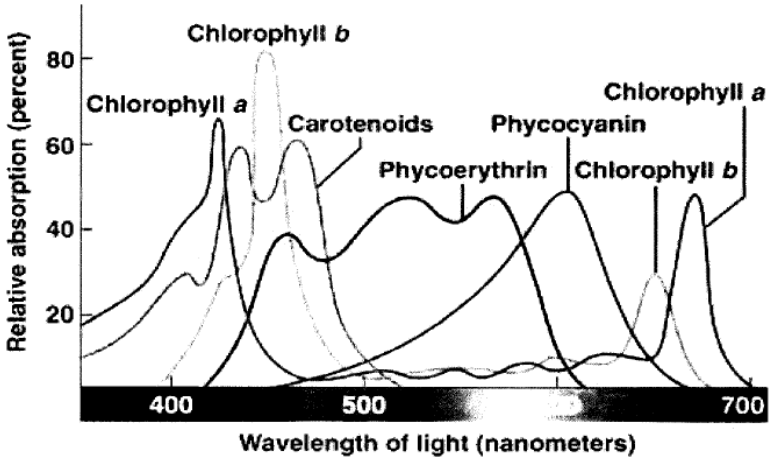

Figure 1.7 The absorption spectrum of photosynthetic pigments (Campbell 2006).

Phycocyanin accounts for about 30% of the biomass (Garnier & Thomas, 1993), but its concentration in the cell depends on environmental growth conditions (Márquez et al., 1995; Márquez-Rocha, 1999). Several authors have suggested that phycocyanin may also function as a carbon storage material (Miller & Holt, 1977) or as a nitrogen source during nitrogen starvation (Boussiba & Richmond, 1980). Among cyanobacteria, the species of genus *Spirulina* are a rich and inexpensive source of this pigment. C-phycocyanin is the major phycobiliprotein in *Spirulina* and may constitute up to 20% of the dry weight of *Spirulina* (Jaouen et al., 1999; Vonshak, 1997). The cyanobacterium, *S. platensis* is an excellent source of phycocyanin. The protein fraction may contain up to 20% of phycocyanin (Vonshak, 1997). In addition, cyanobacterial pigments comprise the most colorful and attractive components in these microorganisms. Screening programs all over the world have further confirmed the diversity and rich repertoire of pigments, which can revolutionize the industrial uses of "colours" with their nutraceutical and pharmaceutical value (Prasanna et al., 2007). The great demand on biological sources of natural colours due to importance of natural colours especially food and cosmetic colourants has started by growing awakening. Cyanobacteria and algae possess a wide range of coloured components including carotenoids, chlorophyll and phycobiliproteins (Henrikson, 1989). Phycocyanin is also used as colourant in food (chewing gums, dairy products, ice sherbaths, gellies etc) and cosmetic such as lipstick and eye liner in many countries. It is also a potential therapeutic agent in

oxidative stress-induced diseases (Minkova et al., 2003). Phycocyanin has significant antioxidant, anti-inflammatory, hepatoprotective and radical scavenging properties. It was also shown to have therapeutic value (immunomodulating activity and anticancer activity). Owing to its fluorescence properties it has gained importance in the development of phycofluor probes for immunodiagnostics (Kronik & Grosmann, 1983). It was used as biomarkers (Herrera et al., 1989), and in cancer laser therapy (Cai et al., 1995).

1.6 Metal Effect

Magnesium

Magnesium (Mg) is an essential macronutrient and has important physiological and molecular roles in plants, such as a structural constituent of chlorophyll molecule, a cofactor for many enzymatic processes associated with phosphorylation, dephosphorylation, and the hydrolysis of various compounds, and a bridging element for the aggregation of ribosome subunits necessary for protein synthesis (Marschner, 1995). As is known, Mg, contained 2.7% molecular weight of chlorophyll, is an important element of chlorophyll and necessary for chloroplast structure (Huber & Maury, 1980). Therefore, Mg plays a fundamental role in both the 'light' and 'dark' reactions of photosynthesis which is driven by apparatus of PS II and PS I which include Chl molecules (Shaul, 2002).

Many investigations which established on concentration of Mg^{2+} in chloroplast showed that its precise level is important for the stacks of thylakoid membranes to form grana (Liang et al., 2009). The grana stacks, which are helpful to transfer energy between chromatophore pigments, are the photosynthetic organs gathered closely, and convert the light quantum to chemical energy efficiently. Additionally, Mg^{2+} has an important contribution on decreasing the damage of chloroplast under stress circumstance and maintaining the normal function of photosynthetic membrane (Huber & Maury, 1980). Mg^{2+} also could obviously elevate the electron transfer rate of shade plant and heliophyte under saturated light intensity (Liang et al., 2009). The mechanism of photosynthetic electron transport affected by Mg^{2+} is

fairly complicated. Under saturated light intensity, the enhancement of the electron transfer rate by Mg^{2+} is due to activation of the PSII reaction center. It is possible that Mg^{2+} can maintain the conformation of antenna pigment, reaction center and some electron carrier of PSII at the molecular level and keep close connection between them, which is helpful to absorb, transfer and transform light energy efficiently (Gupta & Berkowitz, 1989). Many experiments proved that Mg^{2+} could regulate the distribution of excitation energy and the fluorescence yield ratio of PSII and PSI. Liang et, al. (2009) indicated that Mg^{2+} could promote the transfer of light harvesting protein–pigment complex from stroma lamella to enriched-PSII grana, and severing as surrounding antenna of PSII reaction center. Those lead to the increase of light harvesting area of PSII, and the situation is favorable for the excitation energy distribution to PSII.

Both Mg deficiency and excessive supply Mg may be destructive on photosynthesis. Furthermore, the rate of photosynthesis is severely reduced in leaves of Mg-deficient plants as that have been reported in many studies (Fischer, 1997; Sun & Payn 1999; Ridolfi & Garrec 2000). The well-documented effect mechanism of Mg as the central atom of Chl is also the development of chlorosis, typically interveinal, under Mg deficiency stress (Marschner & Cakmak, 1989; Hermans et al. 2004).

In addition photosynthetic role as being central atom of the Chl *a* molecule and structural requirement for chloroplasts, as well as in activating many enzymes, Mg is more responsible for functioning as it excepted than any other mineral nutrient (Epstein & Bloom 2004). Magnesium is, therefore, involved in numerous physiological and biochemical processes in plants affecting growth and development. Some examples of Mg-activated enzymes include ATPases, ribulose-1,5-bisphosphate (RuBP) carboxylase, RNA polymerase and protein kinases (Marschner, 1995; Shaul, 2002). Chlorophyll formation have a process in which to insertion of Mg^{2+} into the porphyrin structure is catalyzed by Mg^{2+}-chelatase that is one of the many key chloroplast enzymes are importantly affected by small variations in Mg levels (Shaul, 2002). Resulting of enzymes effects on carbohydrate metabolism,

deficiency of Mg is likely to decrease the efficiency of Calvin cycle, reduce the utilization of reductive power (NADPH) and cause over-saturation of photosynthetic electron transport system. Under such highly reduced condition, electrons could pass-on to O_2 generating O^{2-} and other ROS (Asada, 2006; Mittler, 2002).

Copper

The ecosystems are subject to various kinds of pollution, having their survival at risk. The release of major pollutants like heavy metals as a result of different human activities into the environment can be highly toxic and this irreversibly damages the ecosystem, and has a great threat to the health of the living beings due to their non-biodegradability and hazardous characteristics (Sheg et al., 2004; Nogales et al., 2011). About 40 heavy metals (HMs) of all (Sandau et al., 1996), pose gradually toxic affecting on humans, animals, plants, and microorganisms; their high concentrations are accumulated in air, soil, and water, being inserted in the food chains (South & Whittick, 1987; Moreno Sa´nchez & Dewars, 1999; Natalya et al., 2003). Heavy metals from wastewater or through the food chain are directly acquired by aquatic organisms like unicellular algae which are an important component of aquatic ecosystems due to their own diurnal metabolic production of oxygen which is required for respiration of other organisms. In addition, photosynthetic organisms like microalgae are the main entrance of heavy metals to food chains, accumulating and attaining pollutants to consumers, including animals and humans, continuously (South & Whittick, 1987; Sandau et al., 1996; Moreno Sa´nchez & Dewars, 1999).

However, some HMs are essential nutrient elements for any plant organism at low doses and these are cofactors of enzymes used in a wide range of biochemical reactions catalyzed by plastocyanin, polyphenol oxidase, superoxide dismutase, ascorbate oxidase, and diamine oxidase and required as components of β-group vitamins and inserted in the carbohydrate and protein metabolism (Lebedev, 1988; Udel'nova & Yagodin, 1993; Bityutskii, 1999). Among heavy metals, copper as a micronutrient is especially involved in the enzymatic redox reactions. Although metals are essential elements at low levels, they can irreversibly damage

physiological and biochemical processes including photosynthesis, respiration, enzyme activity, pigment synthesis in cells, and cell division at concentrations above certain limits. However, it was found that excessive amounts of copper suppress growth of microalgae populations, increasing the lag-phase (Osokina et al., 1986; Bilgrami & Kumar, 1997; Klochenko & Medved, 1999).

Particularly, copper is an essential element for the development of photosynthetic cells but many studies focused on the toxic effects of it. It was reported the decrease in chlorophyll and accessory pigments contents (Rijstenbil et al., 1994) and reduction in growth rate (Laube et al., 1980; Prasad et al., 1998). The effects of toxic copper concentrations on microalgae have been reviewed and it is known that the free cation (Cu^{2+}) performs the toxic effects (Rai et al., 1981; Lobban & Harrison, 1994; Gledhill et al., 1997; Pinto et, al., 2003). However, most species of cyanobacteria have different degrees of susceptibility to toxic copper concentrations by showing several adaptive responses which reduce the damage induced by oxidative stress of copper (Baos et al., 2002; Yan & Pan, 2002). The differential metal effects on different microalgae species result with different adaptive responses. Quigg et al., (2006) reported that smaller cells relating to metal tolerance are more sensitive to copper exposure due to their greater surface/volume ratio. However, metals (including copper) can lead to differential effects on algae species (and even strains within the same species) (Kessler, 1986; Baos et al., 2002; Yan & Pan, 2002), that is reason of significantly varying metal tolerance between different cyanobacteria species (Kessler, 1986; Baos et al., 2002; Yan & Pan, 2002).

CHAPTER TWO
MATERIALS AND METHODS

2.1 Cyanobacterial Culturing Conditions

The cyanobacterium, *S. platensis* (Gamont) Geitler 1952 was used in this study. The organism was provided from Çanakkale 18 Mart University, Faculty of Aquaculture. To prepare and maintain the inoculums, Zarrouk's medium (Table 2.1) was used (Zarrouk, 1966). The utilized carbonate-bicarbonate buffer gives a pH of 9.0 ± 0.2. *S. platensis* was cultivated in batch cultures containing 750 mL of medium at 30 ^{o}C. Also, BG 11 medium was used for *S. platensis* (Table 2.2; Allen, 1968). In growth conditions of *S. platensis*, 0-4.057 mM Mg and 0-5 μM Cu concentrations were used. Culture was inoculated to an initial optical density (600 nm) of ca. 0.2. The cultures were mixed and bubbled using filtered air continuously. Illumination at 2500 lux light intensity was provided by white fluorescent lamps. The light intensity was measured by a digital light meter (Luxtron LX-101). All the reagents used were of analytical grade.

Table 2.1 The Zarrouk medium composition.

Compound	**g/L**
$NaHCO_3$	18 g
$NaNO_3$	2.5 g
K_2SO_4	1.0 g
NaCl	1.0 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
Na_2EDTA	0.08 g
$CaCl_2$	0.04 g
$FeSO_4.7H_2O$	0.01 g
Trace Element solution	1 mL

Table 2.1 The Zarrouk medium composition (continue).

Trace Element A Solution Compounds	**g/L**
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.8
$ZnSO_4.7H_2O$	0.22
$CuSO_4$	0.08
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.02
Vitamin B_{12}	$5x10^{-6}$

Table 2.2 BG11 medium composition

Compounds	**g/L**
$NaNO_3$	15.0
KH_2PO_4	4.0
$MgSO_4.7H_2O$	7.5
$CaCl_2.2H_2O$	3.6
Citric Acid	0.6
Tris	10.0
Ammonium ferric citrate	2.67
Na_2EDTA	0.1
Na_2CO_3	2
Trace element solution	1 mL

Trace Element Solution Compounds	**g/L**
H_3BO_3	2.86
$MnCl_2.4H_2O$	0.05
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.39
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

2.2 Measurement of Optical Density (OD) and pH

The OD was measured spectrophotometrically at the wavelength of 600 nm diurnal. The pH value was measured by a pH meter when the samples were taken daily.

2.3 Dry Weight Determination

The algal suspension was pipetted of homogenous 1 mL volume from growth medium periodically depending on incubation days and dried on an evaporating dish, which of tare is recorded before, at 105^{o}C till drying (at least two hours). Then, this is put in a desiccator and kept in until decreasing its temperature to room conditions. Finally, it was weighted and calculated dry matter.

2.4 Determination of Protein Levels

The cells were harvested periodically depending on incubation days by centrifugation (12000 x g, 10 min, at 4^{o}C), washed with distilled water. The precipitated cells were weighted and 50 mM phosphate buffer, pH 7, added by a rate 12.5 mL for 1 g cells. The cells were homogenized at 8000 rpm 1 min and 9500 rpm 1 min with 30 seconds intervals. Cell debris was removed by centrifugation at 12000 x g, at 4^{o}C for 10 min. The obtained supernatants were used to estimation of protein levels by the method of Bradford using bovine serum albumin as standard (standard function was y=0.0057x, R^2: 0.9831) (Bradford, 1976).

2.5 Determination of Phycobiliprotein Levels

Phycobiliprotein level was determined by the method of Patel et al. 2005. The cells were harvested periodically depending on incubation days by centrifugation (12000 x g, 10 min, at 4^{o}C), washed with distilled water. The precipitated cells were weighted and phosphate buffer, pH 7, added by a rate 12.5 mL for 1 g cells. The cells were homogenized at 8000 rpm 1 min and 9500 rpm 1 min with 30 seconds intervals. Cell debris was removed by centrifugation at 12000 x g, at 4^{o}C for 10 min. The obtained supernatants were used to determination of phycobiliprotein contents. The absorbance was measured at the wavelengths of 562, 620 and 652 nm. The concentrations of phycobiliproteins (mg.mL^{-1}) in the extracts were calculated using the fallowing formulae;

$$[C\text{-}PC] = (A_{620} - 0.474 * A_{652})/5.34$$

$$[APC] = (A_{652} - 0.208 * A_{620})/5.09$$

$$[PE] = (A_{562} - 2.41 * [CPC] - 0.849 * [APC])/9.62$$

2.6 Determination of Chlorophyll a

Levels of chlorophyll *a* were measured as described by Lichtenthaler & Wellburn, (1983). The algal suspension was pipetted by a sterile pipette as being 5 mL from growth medium. The cells in 5 mL suspension were harvested periodically

depending on incubation days by centrifugation (12000 x g, 10 min, at 4^{o}C), washed with distilled water. The precipitated cells were weighted, and then ethanol 95% were poured out in 5 mL volume and mixed slightly. The cells were homogenized at 8000 rpm 1 min and 9500 rpm 1 min with 30 seconds intervals. Cell debris was removed by centrifugation at 12000 x g, at 4^{o}C for 10 min. The obtained supernatants were used to determination of chlorophyll *a*. The absorbance was measured at the wavelengths of 470, 648.6, 664.2 nm. The concentrations of Chl *a* (µg.mL^{-1}) in the supernatants were calculated using the fallowing formulae;

$$[\text{Chl } a] = 13.36*A_{664.2}-5.19*A_{648.6}$$

2.7 Determination of Proline (Pro) Content

Proline content was assayed by the method of Bates et al., (1973). The cells were harvested periodically depending on incubation days by centrifugation (12000 x g, 10 min, 4^{o}C), washed with distilled water. The precipitated cells were weighted and 3% sulfosalicylic acid, added by a rate 10 mL for 1 g cells. The cells were homogenized by 8000 rpm 1 min and 9500 rpm 1 min with 30 seconds intervals. Cell debris was removed by centrifugation at 12000 x g, at 4^{o}C for 10 min. 2 mL ninhydrine, 2 mL glacial acetic acid and 2 mL of obtained supernatant were mixed in a glass tube. The mixture was incubated in hot water bath at 100^{o}C for 1 hour. Then the tube is hold in an ice bath and added 4 mL toluen. The recent mixture is vortexed for 15 seconds and the absorbance was measured at 520 nm. The concentrations of proline (µmol.g fresh matter^{-1}) in the extracts were calculated using the fallowing formulae;

$$[\text{Proline}]=[\ (\mu g \text{ proline/mL} * \text{toluene}) / 115.5\ \mu g\ /\text{mol}] / [(\text{g sample})/5]$$

2.8 Determination of Lipid Peroxidation (LPO)

LPO was estimated based on thiobarbituric acid (TBA) reactivity. Samples were evaluated for malondialdehyde (MDA) production using a spectrophotometric assay for TBA (Ohkawa et al., 1979). The cells were harvested periodically depending on

incubation days by centrifugation (12000 x g, 10 min, 4^{o}C), washed with distilled water. The precipitated cells were weighted and 5% trichloroacetic acid (TCA), added by a rate 1.5-2 mL for 0.1 g cells. The cells were homogenized by 8000 rpm 1 min and 9500 rpm 1 min with 30 seconds intervals. Cell debris was removed by centrifugation at 12000 x g, 4^{o}C for 10 min. Equal volumes of supernatant and 0.5% TBA (TBA solved in 20% TCA) were mixed in a glass tube. The mixture is incubated in hot water bath at 95^{o}C for 30 minutes. Then the tube is hold in an ice bath and centrifuged at 1000 rpm for 5 minutes. Then the absorbance was measured at the wavelengths 532 and 600 nm. The concentrations of MDA (nmol.g^{-1} fresh matter) in the extracts were calculated using the fallowing formulae;

$$[MDA]=[(A_{532}-A_{600}) * \text{volume of extraction}] / [155 * \text{g sample}]$$

2.9 Metal Analysis

The concentrations of the metals were measured by inductively coupled plasma optical emission spectrophotometer (ICP-OES, Optima 2100DV, Perkin Elmer, USA). 1 g wet weight of *S. platensis* cells was digested in 2 mL nitric acid followed by 2 mL perchloric acid. All glassware washed with 0.1 M nitric acid and ultra-pure water before use.

2.10 Photosystem II Activity

Thylakoids were prepared in 0.5 M sucrose, 0.5 M K-phosphate and 0.3 M sodium citrate all at pH 7.0. This is called SPC buffer. The cells were harvested periodically depending on incubation days by centrifugation (12000 x g, 10 min, 4^{o}C), washed with distilled water. The precipitated cells were weighted and SPC buffer, added by a rate 5 mL for 1 g cells. The cells were homogenized by 9500 rpm half minute with 10 seconds intervals. Cell debris was removed by centrifugation at 2000 x g, at 4^{o}C for 5 min. Supernatant was centrifuged again at 4250 x g, 10 min and the pellet, inwhich tkylakoids are precipitated, is used. Supernatant is used to calculate to chlorophyll level of cells as described before. Pellet is diluted by SPC buffer by a rate 5 mL for 1 g. precipitated cell and added to reaction medium in a

volume to get a certain concentration to measure PS II activity by method of Henriques (2004). PS II dependent electron transport activity was measured at room temperature with a recording spectrophotometer by following the bleaching of 2,6-dichlorophenol indophenol (DCIP) at 580 nm using water as electron donor. The standard assay medium contained 50 mM Hepes (pH 7.5), 0.3 M sucrose, 10 mM NaCl, 2 mM $MgCl_2$, 20 μM DCIP. Thylakoids were added to a concentration of about 20 μg chlorophyll per milliliter and an extinction coefficient of 19 800 M^{-1} cm^{-1} was used to calculate the rate of DCIP reduction.

2.11 Determination of Carotenoid Content by HPLC

S. platensis cells were harvested periodically depending on incubation days by centrifugation (12000 x g, 10 min, 4^oC), washed with distilled water. The precipitated cells were weighted and chloroform added by a rate 5 mL for 1 g cells. The homogenization was done as mentioned above. The homogenizate was centrifuged after that supernatant filtered by ultrafilters (0.2 micron). Filtered supernatant was injected as 20 μL and applied HPLC assay by a tertiary phase given on the Table 2.3. Carotenoids, β- carotene, β-cryptoxanthin, Zeaxanthin and Lutein **s**tandards were prepared both in water and chloroform prior to analysis to decide solvent of crude extract and evaluate to retention times. All samples were prepared in chloroform due to well solubility of carotenoids in it. HPLC system used for carotenoids content assay was the method of Kraay et, al., (1992). HPLC system used was an Agilent Technologies 1100 series. The reversed phase column was a 250x 4.6 mm ACE-221-2546 C18 column. The column was maintained at a constant temperature of 32 °C using a column thermostat (Agilent Technologies 1200 series G1316A TCC). The gradient was based upon a ternary solvent system (Table 2.3). All solvents were degassed nanograde HPLC solvents. Solvent A consisted of 85% methanol/water (v/v), buffered with 0.5 M ammonium acetate (final concentration), solvent B consisted of 90% acetonitrile/water (v/v) and solvent C of ethyl acetate. The flow rate was kept at 0.8 mL/min. The column was equilibrated prior to use by flushing with 60% mobile phase B (v/v) for 5 min. Sample volumes were injected under the control of an external personal computer that supported chem. station software. Pigments were detected by diodearray spectroscopy (wavelength range:

400 to 750 nm, 1.2 nm spectral resolution). Data were recorded and processed by Chemstation software (peak identification on the basis of retention time in combination with comparison with a spectral library). The detection limit of the system is 0.01 $mg.L^{-1}$. The coefficient of variation of replicate injections was typically <1%. A suit of standards was always processed with every batch of samples.

Table 2.3 The solvent gradient that was applied for chromatography (Kraay et al., 1992; solvent A: 85% methanol/water (v/v), buffered with 0.5 M ammonium acetate (final concentration), solvent B: 90% acetonitrile /water (v/v), solvent C: ethyl acetate)

Time (min)	**% A**	**% B**	**% C**
0	60	40	0
2	0	100	0
7	0	80	20
17	0	50	50
21	0	30	70
28.5	0	30	70
29.5	0	100	0
30	60	40	0
35	60	0	0

Following standard graphics of carotenoids were given. The calculations of carotenoids were made by using standard graphic functions (Figure. 2.1).

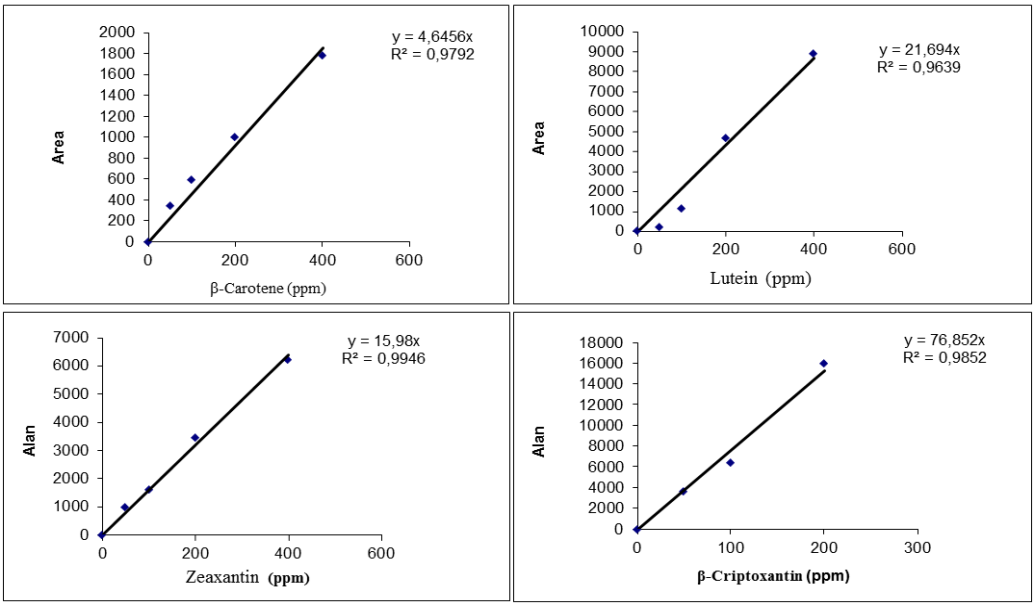

Figure 2.1 Standard graphics of carotenoids.

CHAPTER THREE
RESULTS AND DISCUSSION

Growth medium, in which the cyanobacterium was thrived, is the most important parameter for investigation. Therefore, different media were assayed on preliminary experiments. In the literature, the most common media, which are used to grow of *S. platensis*, were BG11 and Zarrouk's medium. Optimum growth was dedicated in Zarrouk's medium so that during all experiments it used. Similarly, industrial production of *S. platensis* usually rely on Zarrouk medium (Belay, 2008). Mg ve Cu as well as temperature effects (37^{o}C) on OD_{600} changes, dry biomass, pH, protein, CPC, APC, chlorophyll, proline production, PS II activity and LPO levels in *S. platensis* were investigated in the Zarrouk's growth medium included Mg (0-4.057 mM) and Cu (0-5 μM) concentrations during the incubation period. The obtained results were compared with their controls values.

3.1 Effects of Magnesium or Copper Concentrations on the Growth, Dry Biomass, Protein and pH Levels in *S. platensis*

In the growth media containing Mg and Cu, growth rate of *S. platensis* was determined with optical density and dry biomass values depending on incubation time (Figures 3.1 and 3.2).

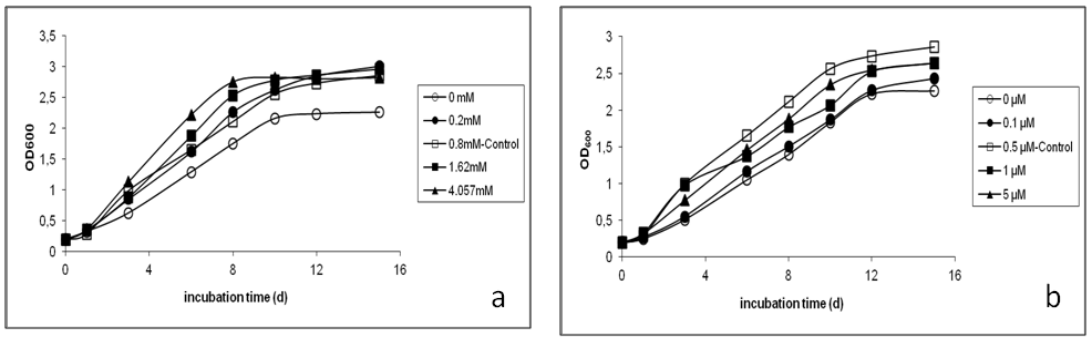

Figure 3.1 Variations of OD in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

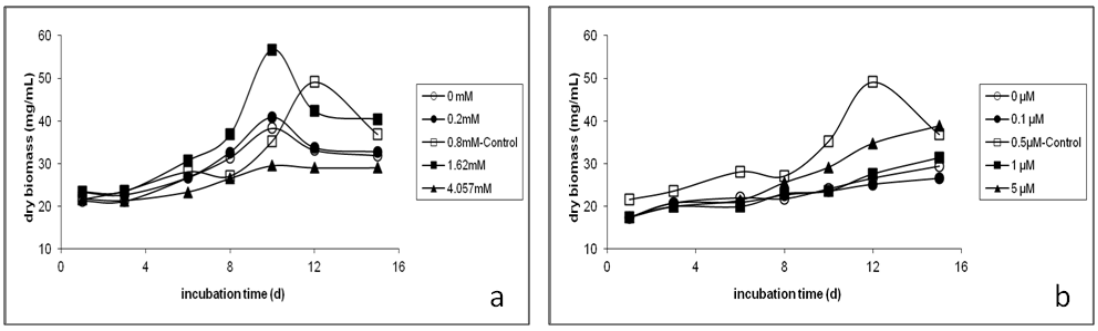

Figure 3.2 Variations of dry biomass in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

As seen on Figure 3.1.a; maximum growth of *S. platensis* was on 10th day in the medium containing 4.057 mM Mg. It was also determined non-significant changes on growth of *S. platensis* between 1.62 mM and 4.057 mM Mg concentrations. Then the OD values was not much changed excluded Mg absence condition. At Figure 3.2.a; the highest dry biomass value was determined in the medium containing 1.62 mM Mg on 10th day as 56.7 mg mL^{-1}, following a decrease on 12th day obtained and then remained stable. On 12th day, maximum dry biomass was obtained at control condition as 49.1 mg mL^{-1}. The variations in dry biomass at concentrations of 0 and 0.2 mM were showed same trends by reaching to maximum on 10th day and following a decrease. The obtained biomass values were 38.3 mg mL^{-1} and 40.8 mg mL^{-1}, respectively.

As shown in Figure 3.2.b, the highest biomass determined in control condition including 0.5 µM Cu independently to the cultivation time namely on any day the highest OD value were found at 0.5 µM. However, after 12th day similar OD values were obtained in presence 1 and 5 µM Cu concentrations. The dry biomasses were increased depending on cultivation time and the highest value was determined as 49.1 mg mL^{-1} in 0.5 µM Cu. The biomass at excess Cu concentration (5 µM) was determined as 34.7 mg mL^{-1} that is lower than control condition. It may result in a decrease due to high concentration Cu. Excess concentrations of Cu might suppress to growth. Also, at deficient (0.1 µM) or lack (0 µM) Cu conditions, the dry biomasses were under control value nearly half fold due to its necessity to growth at certain concentration. As illustrated in the 3.2.b the dry biomasses were stayed

constant until 6th day, after 6th day had been slightly increasing trend although the values about half fold of control value and the increases were continued during incubation period. It might be said that the lag and exponential phases of growth were prolonged when compared with control Cu concentration.

In the growth medium containing Mg of *S. platensis*, the highest protein content was on 10th day for the tested concentrations (Fig 3.3.a). In the medium containing Cu; the highest protein content was obtained on 10th day (Fig 3.3.b). Absence of Mg in the medium was showed negative effect on the protein level. It may attributed to that Mg is essential element which play role in many enzymatic system related protein synthesis. However, varying concentrations of Mg caused to different levels protein. Cu is necessary as micronutrient for protein metabolism. It may be sensitive for protein synthesis. *S. platensis* was had higher protein content at the control concentration (0.5 µM Cu). It might also be said that protein accumulation was decreased at the lack of Cu condition. Although protein accumulation was induced by certain concentration of Cu in the medium, the higher Cu conditions may be caused to damage protein molecules creating ROS (Halliwell & Gutteridge, 1999).

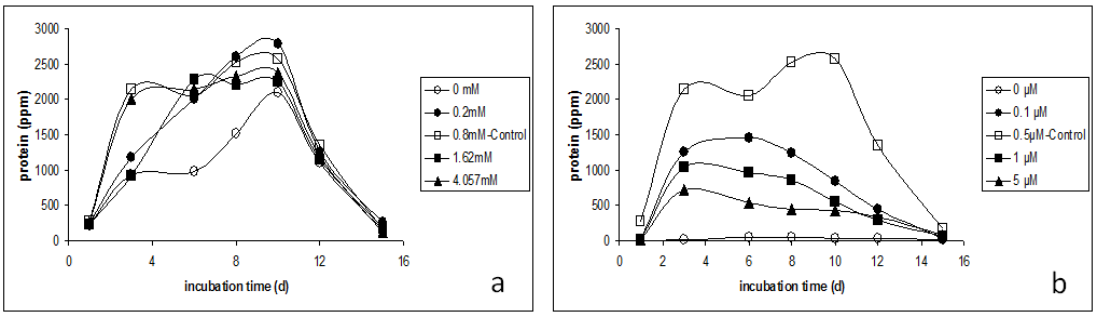

Figure 3.3 Variations of protein content in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

In the medium containing Mg (Figure 3.4.a); pH values depending on its concentrations were very similar and varied from 9 to 10.7. But as shown on Figure 3.4.b, in the medium containing Cu; at control concentration 0.5 µM Cu, pH was about 10.5 on 8th day then slightly increased and then remained stable. In the other tested Cu concentrations pH levels had similar trend.

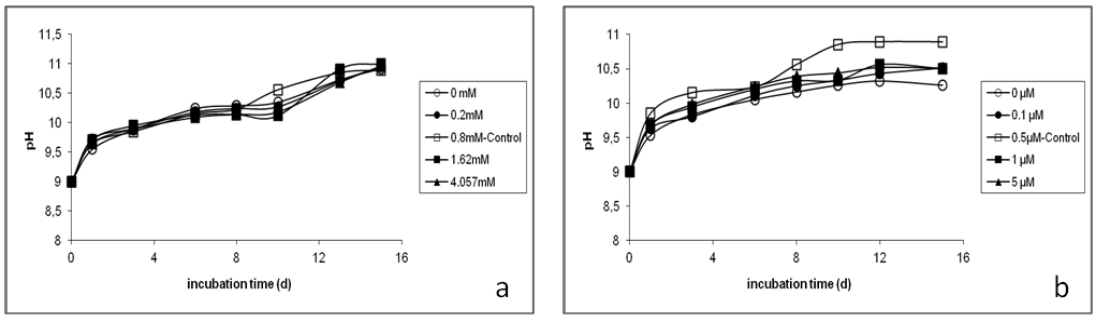

Figure 3.4 Variations of pH values depending the incubation period in *S. platensis* medium containing Mg (a) or Cu (b) concentrations.

3.2 Effects of Magnesium or Copper Concentrations on the Chlorophyll a Productions in *S. platensis*

As depicted in Figure 3.5; changes in chlorophyll *a* levels of *S. platensis* depending on the Mg or Cu concentrations are showed during incubation time.

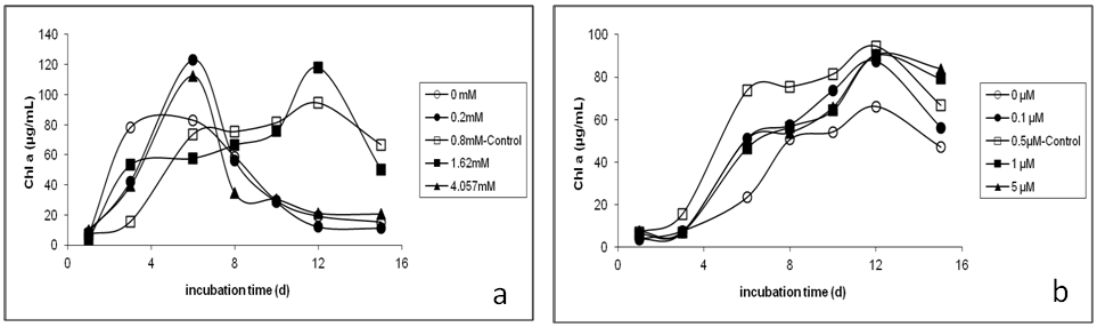

Figure 3.5 Variations of Chl *a* values in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

In the medium containing Mg (Figure 3.5a); the maximum Chl *a* levels were obtained on 12th day for 1.62 mM and 0.8 mM (control) Mg as 118.1 and 94.4 µg mL^{-1} respectively. However, the highest Chl *a* level was determined in 0.2 mM Mg condition on 6th day as 123 µg mL^{-1}. In the medium containing Cu (Figure 3.5b); the maximum Chl *a* values were obtained on 12th day for all tested Cu concentrations. The levels of Chl *a* were decreased at 0.2 mM and 0 mM Mg after the 6th day as respected due to dry biomasses at these concentrations were under control conditions. This might be attributed to that Mg is an essential nutrient and deficiency or absence of it was not contributed to an increasing on dry biomass. An increment in

dry biomass may result a rising trend in Chl *a*. Also, levels of Chl *a* of *S.platensis* were affected by excessive Mg concentration (4.057 mM) showing constantly decreased then remained constant as shown in decreasing of dry biomass at this concentration. It was not made a major contribution on dry biomass at this concentration. However, levels of dry biomass and Chl *a* were showed similar trend at control condition (0.8 mM) Mg, the maximum values were on 12^{th} day as 49.1 $mg.mL^{-1}$ and 94.4 $\mu g.mL^{-1}$ respectively. After 6^{th} day, the insufficient and absence of Mg caused to decrease in Chl *a* levels. This may be resulted from that Chl *a* molecules may be lost Mg ions in the active center that Chl *a* can normally be degraded into pheophyin a (derivative of Chl *a* without Mg ion), but in the absence of Mg in the medium, this conversion increased. For production of higher Chl *a* level thoughout incubation time, Mg and Cu concentrations were 0.8 mM and 0.5 µM respectively. At higher Cu concentrations, Mg may be replaced by Cu so that Chl *a* degraded. In algae exposed to high copper concentrations, decreases in chlorophyll pigment levels have been reported (Schiariti et al., 2004).

3.3 Effects of Magnesium or Copper Concentrations on the Carotenoids and Phycobiliprotein Levels of *S. platensis*

Table 3.1 shows, HPLC results of carotenoids which produced by *S. platensis* on 12^{th} day. Based standard chromatograms, the retention times were 11.8, 12.2, 18.5 and 22.5 minutes for Lutein, Zeaxanthin, β-Cripthoxanthin and β-Carotene, respectively. Carotenoid accumulation was observed with increasing concentration of Mg. The positive effect of divalent cations might be attributed to a stimulatory effect on carotenoid-synthetizing enzymes. Similarly, carotenoid composition may be also affected by varying concentrations of Mg (El-Banna et al., 2011). Fang et al., (2010) found that carotenoid production was increased by addition of certain level magnesium sulfate into the medium. The highest levels of carotenoids were obtained in absence of Cu condition. β-Carotene was the major carotenoid pigment of *S. platensis*. The results shown in table indicate that effect of increasing concentration Cu on the carotenoids was observed as a gradually decrease on their levels. The decrease of carotenoid levels with Cu concentration is consistent with results of

Deniz et al., (2011). It may be attributed to toxic effect of Cu at high concentrations. Pigment synthesis might be inhibited and pigment degradation was increased.

Table 3.1 Carotenoids levels of *S. platensis* by varying concentration of Mg (a) or Cu (b) on 12th day

µg/mL a	Influence of varying concentrations of Mg on carotenoid levels of *S. platensis* on 12th day				
	0 mM	0.2 Mm	0.8 mM-cont	1.62 mM	4.057 mM
β-Carotene	1464.1	1822.28	2013.31	2354.15	2299.43
β-Cripthoxanthin	455.11	669.61	681.45	713.57	719.14
Zeaxanthin	448.12	674.04	644.23	700.09	705.96
Lutein	156.52	194.04	201.55	215.87	208.64

µg/mL b	Influence of varying concentrations of Cu on carotenoid levels of *S. platensis* on 12th day				
	0 µM	0.1 µM	0.5 µM-cont	1 µM	5 µM
β-Carotene	2396.73	2253.24	2013.31	1453.66	1211.85
β-Cripthoxanthin	900.44	765.25	681.45	536.87	412.48
Zeaxanthin	821.66	703.99	644.23	495.81	445.28
Lutein	214.08	223.78	201.55	232.88	121.05

Phycobiliproteins are important accessory pigments in *S. platensis*. These consist of CPC, APC and PE. Highest CPC was recorded in *S. platensis* on 10th day in both cultures (Fig 3.6.a. and 3.6.b.). CPC was one of the major pigment fractions in *S. platensis* which grows in all the media contained Mg concentrations (Fig 3.6.a.) and the CPC contents were approximately similar with their maximum levels on 10th day. It is also curious about OD values on the upper or lower conditions were not significantly greater, suggesting that growth conditions were not still limited by Mg levels. However, in the media containing Cu; the highest CPC value was obtained at 0.5 µM Cu control condition in all incubation period as 1967 µg mL^{-1}. In the *S. platensis*, the 20% of dry weight fraction is constituted by phycobiliproteins (Vonshak, 1997).

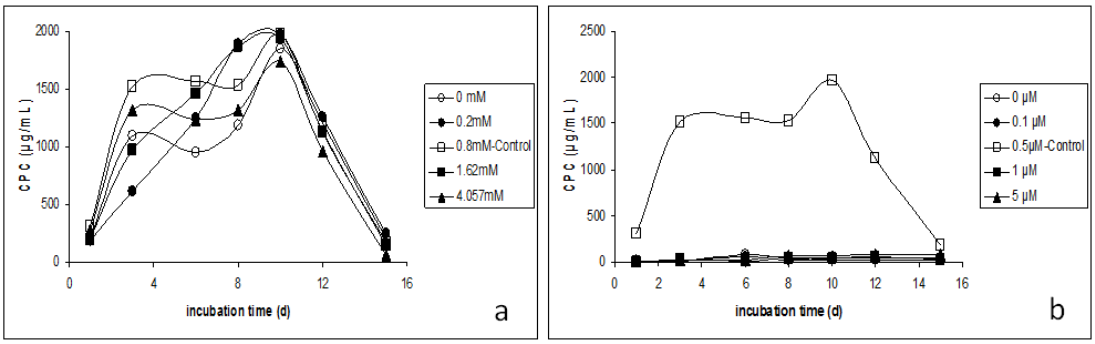

Figure 3.6 Variations of CPC values in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

The synthesis of phycobiliproteins was affected by Cu concentration as became a cofactor (Jaouen et al., 1999; Vonshak, 1997). It may be understood that the levels of phycobiliproteins were low in presence of 1 and 5 µM Cu due to its toxic effects that is expected when present in excess. This was supported by Hemlata Tasneem, (2009) who explained that the decline in phycobiliprotein content depending on varying metal toxic concentrations. Phycobiliproteins in 0 and 0.1 µM Cu conditions were produced very lower compared to control values due to its necessity as a trace metal. Thus, absence of Cu was also caused negative effects on CPC and APC productions. Tredici et al., (1986) have underlined in their study that physiological stress induced by cultures of *Spirulina maxima* is resulted in a reduced rate of protein synthesis, an increase in the carbohydrate content, a massive fragmentation in the trichomes and strong reduction in the phycobiliprotein content. However, cell concentrations in these conditions were not very different from values obtained in control cultures (0.5 µM).

As depicted in Figure 3.7.a; APC was reached to highest value as 567 µg mL^{-1} on 10^{th} day of incubation. As shown in Figure 3.6 and Figure 3.7, APC was major pigment fraction along with CPC and their content showed similar trend but CPC level was higher than APC level during cultivation period. In content of APC, there was a rising trend with incubation period in varying Mg concentrations. The highest level of APC was observed on the control condition of Cu (Fig 3.7.b.). However, the levels of APC were followed a similar trend with CPC levels during incubation period in both metal media. While CPC, APC pigments were produced very higher

levels in *S.platensis* cultures, PE was insignificantly produced so it was not depicted here.

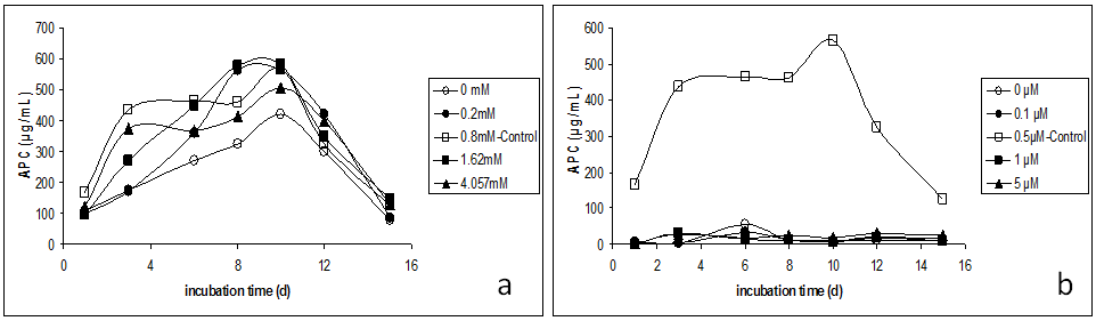

Figure 3.7 Variations of APC values depending the incubation period in medium *S. platensis* containing Mg (a) or Cu (b) concentrations.

3.4 Effects of Magnesium or Copper Concentrations on the Proline and LPO Levels in *S. platensis*

Proline levels of *S. platensis* depending on the Mg and Cu with respect to incubation time are depicted in Figure 3.8.

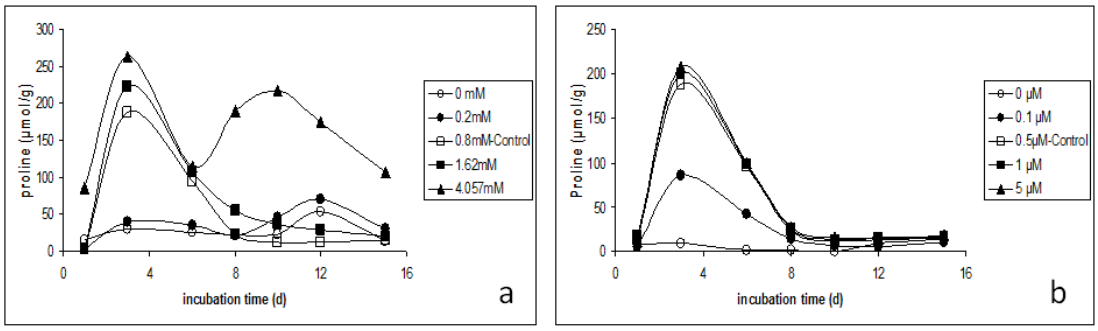

Figure 3.8 Variations of proline level in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

In the medium containing Mg (Figure 3.8.a); the proline levels were increased depending on gradually rising concentration of Mg (for 0.8 (control), 1.62, 4.057 mM) on 3rd day. On 12th day culture conditions containing 0 and 0.2 mM Mg were observed maximum peaks. The highest proline level obtained on 3rd day as 263.196 µmol g^{-1} in presence of 4.057 mM Mg containing medium. As seen on Figure 3.8.b; at higher Cu concentrations, proline levels were decreased after the 3rd day of

incubation period. On 3rd day; the highest proline content in presence of 5μM Cu was determined. The dry biomasses between 1st and 6th days were not much changed at higher Cu concentrations while proline levels were relatively higher in this period. This result is supported by Choudhary et al., (2007). They showed that proline accumulation increased with rising concentration of the metals. On initial days of cultivations, in both higher concentrations of Cu and Mg, the proline levels were extremely high. It might be attributed that the *S. platensis* cells were on the adaptive period at metal stress conditions. Especially higher concentrations of both metals (Mg or Cu) were caused to higher proline accumulation as leading stress. Stabilization of proteins and protein complexes in the chloroplast and cytosol was provided by proline accumulation during stress. Therefore, it is important as functioning on protection mechanism of the photosynthetic apparatus and enzymes involved in detoxification of ROS (Szabados & Savoure, 2009).

Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation (LPO) and an indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979). Variations of MDA levels in *S. platensis* depending on incubation period in medium containing Mg and Cu concentrations were shown in the following graphics (3.9.a. and 3.9.b.). As shown in our experiments, increase in both proline and MDA contents with increasing metal ion concentration is indicative of a correlation between free radical generation and proline accumulation (Choudhary et al., 2007). Wu et al., (1998) in their results observed proline accumulation in response to Cu^{2+} stress in *Chlorella*. These observations were also supported to our results.

In the growth media containing Mg of *S. platensis*; the highest MDA values were obtained 10th day for 0.8 mM (control) and 0.2 mM. However, the highest MDA values in presence of 1.62 mM and 4.057 mM Mg were on 6th day. In the growth media containing Cu of *S. platensis*; MDA levels of control (0.5 μM Cu) were not extremely changed depending on incubation period. MDA content was increased with a rising at heavy metal concentration in the culture medium. This situation might cause to concentration-dependent free radical generation.

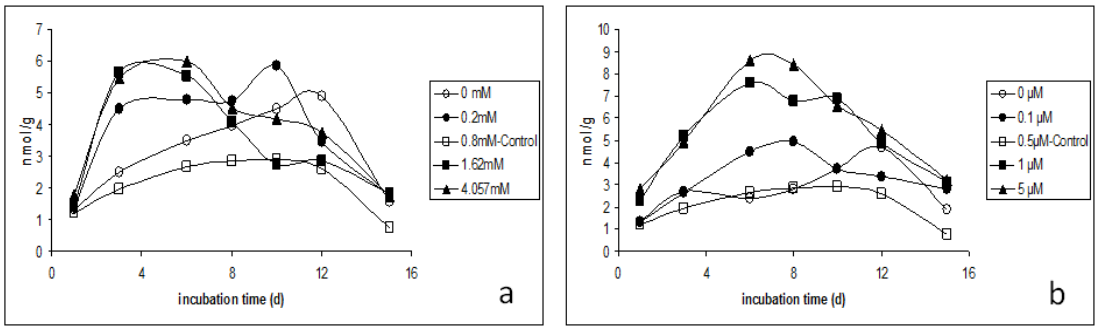

Figure 3.9 Variations of MDA levels in *S. platensis* depending the incubation period in medium containing Mg (a) or Cu (b) concentrations.

The highest MDA level was observed on 6th day for the media containing 5 µM Cu. The MDA levels were showed similar trend for high concentrations of Cu (1 and 5 µM) depending incubation period. The level of MDA is considered as a measure of lipid peroxidation. The effects of higher copper concentrations on growth of *S. platensis* may result higher MDA content when compared the control. Moreover, compared to control, MDA contents were also found higher at different concentration of Mg during incubation period. According to results the highest MDA content was detected in excess Cu concentration due to its toxic effect.

3.5 Effects of Magnesium or Copper Concentrations on the Essential Metal Content of *S. platensis*

As shown in Table 3.2; Entrance of Ca into the *S. platensis* cell was supported in presence of 4.057 mM Mg. For control condition Mg (0.8 mM), the Ca content was constantly increased by the incubation period. On 15th day, the highest content of Ca was observed at 0.8 mM Mg concentration as 95.018 mg g^{-1}. Absence of Mg was increased to entrance of Ca into cells up to 6th day, then it was not much chanced till 10th day and after the 10th day it was showed increasing trend. It may be said that Mg deficiency (0 mM) may be caused to an inductive effect on Ca content so that it increased during incubation period.

Table 3.2 The essential metal content of *S. platensis* depending on different magnesium concentration

	Concentration of Magnesium		
Contents depending of days ($mg.g^{-1}$)	**0 mM**	**0.8 mM-Control**	**4.057 mM**
Ca			
0	0	0	0
6	24.703	57.676	40.569
10	21.000	69.708	43.793
15	47.047	95.018	67.110
Cu			
0	0	0	0
6	0.260	0.316	0.613
10	0.305	0.260	0.204
15	0.276	0.286	0.219
Fe			
0	0	0	0
6	30.502	32.262	40.406
10	27.410	46.896	38.543
15	35.708	73.028	57.430
Mg			
0	0	0	0
6	81.370	92.904	135.855
10	75.200	98.878	138.652
15	53.705	107.505	270.373
Mn			
0	0	0	0
6	1.621	1.533	2.112
10	1.596	2.057	1.909
15	2.315	2.589	2.103

As shown in Table 3.2; the Cu uptake in deficiency of Mg was showed a stable trend throughout cultivation period. At the higher concentration of Mg (4.057 mM), Cu uptake was constantly increased till 6^{th} day, then it was decreased and after the 10^{th} day it was nearly stable. It may be said that increasing of Mg was resulted with increasing of Chl levels and photosynthesis so that necessity of Cu was increased first days. Following days Cu uptake into the *S. platensis* was inhibited due to its toxic effect. At Mg control condition (0.8 mM), Cu content was not much changed with incubation period while the content of Fe was constantly increased. At the higher concentration of Mg (4.057 mM), Fe uptake was constantly increased till 6^{th} day, then it was not much changed and after the 10^{th} day it was increased. Presence of 4.057 mM Mg was increased to entrance of Fe on initial days whereas photosynthetic pigments such as Chl *a*, CPC was produced higher than following days due to necessity of Fe for synthesis of these pigments (Ozturk Urek & Tarhan, 2011). After the 10^{th} day, Fe was showed an increase trend staying under control

value. Inexistence of Mg was caused an increase on the Fe level up to 6th day, after this day it was not much chanced due to total content of pigments (such as Chl *a*, CPC and APC) was not much increased. The results in which Fe-deficient condition was exhibited decreased pigment levels in *S. maxima* were similar (Ozturk Urek & Tarhan, 2011).

As depicted on table 3.2; at control condition of Mg (0.8 mM), the entrance of Mg into *S. platensis* cells was firstly increased and then stayed stabile during incubation period. In the presence of 4.057 mM Mg, Mg uptake was constantly increased and it was reached to a maximum value as 270.373 mg mL^{-1}. The increase of Mg content to maximum value at excessive condition (4.057 mM) may be resulted from being dominant ions in the medium. At 0 mM Mg was slightly increased till 6th day, after this day it was shown a decreased trend. Manganese accumulation in *S. platensis* cells was linearly increased with incubation period at 0.8 mM control condition. It was reached to the highest value on 15th day as 2.589 mg g^{-1}. In the upper control condition (4.057 mM), the entrance of Mn into the cells firstly was increased, then stayed stabile. It may be expected, presence of excessive Mg was blocked to entrance of Mn, and then Mn content balanced. Mn content of *S. platensis* was showed an increment in the early days of incubation period, following a decreasing and then reached a maximum on 15th day at the lack of Mg in the medium. It was attributed to that Mn uptake was occurred instead of Mg and it was reached to a value as 2.315 mg g^{-1}.

Table 3.3 The essential metal content of *S. platensis* depending on different copper concentration

	Concentration of Copper		
Contents depending of days (mg g^{-1})	**0 µM**	**0.5 µM-Control**	**5 µM**
Ca			
0	0	0	0
6	40.075	57.676	90.714
10	31.465	69.708	86.659
15	74.148	95.018	196.644
Cu			
0	0	0	0
6	0.354	0.316	0.282
10	0.214	0.260	0.268
15	0.186	0.286	1.136
Fe			
0	0	0	0
6	45.958	32.262	26.675
10	31.407	46.896	24.801
15	58.138	73.028	131.995
Mg			
0	0	0	0
6	121.298	92.905	78.830
10	51.687	98.878	72.514
15	78.784	107.505	206.643
Mn			
0	0	0	0
6	2.318	1.533	1.353
10	0.984	2.057	1.305
15	1.599	2.589	3.953

As it can be seen on the table 3.3; in the presence of 0.5 µM Cu, Ca content in the *S. platensis* cells was gradually increased by the incubation period. At the higher concentration of Cu (5 µM), Ca level in the cells following a rising trend was reached a value as 196.644 mg g^{-1} on 15^{th} day. Inexistence of Cu condition was observed an increase till 6^{th} day then, decreased and then, increased a value as 74.148 mg g^{-1} stayed under control condition on 15^{th} day. At all concentrations, Cu content in *S. platensis* cells was increased till the 6^{th} day. After 6^{th} day at control condition (0.5 µM), Cu content was slightly decreased and stayed stabile. In presence of 5 µM Cu after 6^{th} day it was decreased and then increased again. At the 0 µM Cu concentration, after the 6^{th} day Cu content was showed slightly decreased trend. As shown in table 3.3; at all Cu concentrations, Fe content in *S. platensis* cells was increased till the 6^{th} day. After 6^{th} day at control condition (0.5 µM), Fe content was constantly increased. In presence of 5 µM Cu after 6^{th} day it was not much changed and then reached a maximum value as 131.995 mg g^{-1} on 15^{th} day. At the 0 µM Cu

concentration, after the 6th day Fe content was decreased then increased again. As depicted in table 3.3; the Mg content in the cells at control condition (0.5 µM Cu) was increased. In presence of excessive Cu (5 µM Cu), the maximum Mg content as 206.643 mg g^{-1} was obtained on 15th day. In deficiency of Cu, it was increased till 6th day then, decreased.

In presence of 0.5 µM Cu, entrance of Mn into the *S. platensis* cell was constantly increased by the incubation periods. On the initial days, lack of Cu (0 µM) was caused high entrance of Mn till 6th day then decreased and after the 10th day it was raised staying under control level. At the excessive concentration of Cu (5 µM), Mn content of cells was increased similar to control up to 6th day then it was not much chanced till 10th day and then reached a maximum value as 3.953 mg g^{-1} on 15th day.

3.6 Effects of Magnesium or Copper Concentrations on the PSII Activities of *S. platensis*

As can be seen in the Table 3.4; the highest PSII activities were determined on control growth medium (0.5 µM Cu and 0.8 mM Mg) as 14.4 U L^{-1} and 18.5 U L^{-1} for the 8th and 10th day, respectively. In the presence of 5 µM Cu, the activity was obtained as 5.6 U L^{-1} on 10th day while it was 4.4 U L^{-1}on 8th day. In the absence of Cu, PSII activities were also decreased. According to this result, excessive Cu concentration was had a toxic effect on the PSII activity. It may be said that the excess Cu may damage to the photosynthetic apparatus and caused reduction of pigment content (Ciscato et al., 1997). At higher Cu concentrations, Mg which is central atom of Chl molecules may replace by Cu atom resulting by losing its function (Küpper et al., 2002). Absence of Cu was also showed negative effect on PSII activity. It was attributed that metal interactions which are required at lumen side of PSII may change related to lack of Cu (Sersen et al., 1997; Miqyass et al., 2007). However, presence of Cu as micronutrient may be essential for all photosynthetic process.

Table 3.4 Changes on PSII activities of *S. platensis* depending on stress conditions by incubation time.

Incubation time	**PS II activities of *S. platensis* (U L^{-1})**			
Cu concentration	**0 µM**		**0.5 µM- control**	**5 µM**
8^{th} day	7.9		14.4	4.4
10^{th} day	9.3		18.5	5.6
Mg concentration	**0 mM**	**0.2 mM**	**0.8 mM- control**	**1.62 mM**
8^{th} day	2.92	5.23	14.4	9.44
10^{th} day	2.32	7.38	18.5	10.52

Decreasing of Mg concentration was caused gradually decreasing on PSII activities. It may be due to necessity of Mg ions biosynthesis of the Chl *a* molecules. The lowest PSII activity was observed at the absence of Mg condition on 10^{th} day. This result may attribute to Chl *a* molecules had been already present in the cells of *S.platensis*. The highest PSII activity which was found at the 0.8 mM Mg concentration was showed that this concentration of Mg might be improved PSII conformation and promoted to energy transfer and water splitting (Liang et al., 2009). At the upper control concentration of Mg (1.62 mM), the PSII activities were detected as 9.44 U L^{-1} and 10.52 U L^{-1} for the 8^{th} and 10^{th} day, respectively. It was seen that the PSII activities at excessive condition (1.62 mM) were higher than that detected at decreased concentration of Mg (0, 0.2 mM). It may be said that Mg in excess treatment might be affected to PSII activity lower when compared to decreased concentration of Mg (0, 0.2 mM). Also, Mg concentration could be affected to interferences between mineral metals around PSII complexes. Changing microenvironment within PSII could be improving pigments bound to protein therefore visible absorbance may increase.

3.7 Effect of Temperature on the Investigated Cyanobacterial Metabolites

Temperature is an important parameter which may increase some metabolite levels while it can decrease some other metabolite levels. The table 3.5 shows effects of temperature changing on investigated metabolites at control condition on 12^{th} day.

Table 3.5 Effect of increasing temperature on investigated metabolites at control condition on 12th day.

	T=30°C	**T=37°C**
OD_{600}	2.785	2.760
Dry Biomass (mg mL^{-1})	49.1	53.8
Chl *a* (µg mL^{-1})	94.4	122.53
CPC (µg mL^{-1})	1187	165
APC (µg mL^{-1})	367	56.97
Proline (µmol g TA^{-1})	18.3	19.43
MDA nmol g TA^{-1})	2.38	12.5
Protein (ppm)	1363	519

OD_{600} values at 30 and 37 °C were very close on 12th day. The dry biomass which measured at 37°C was higher compared with value of obtained at 30 °C as showing positive effect. CPC and APC levels were significantly decreased with increasing temperature while Chl *a* level increased. Structural stability of phycobiliproteins may be extremely affected from temperature. The increment of Chl *a* level may be result of adaptation to increasing energy. Proline contents also were similar. When the temperature was increased to 37 °C, the protein level was decreased. MDA content was detected approximately 5 fold higher at 37 °C on 12th day. This indicated that the temperature of 37 °C was caused to stress on *S. platensis* cells.

In addition, the effect of temperature on PSII activity at the control condition (0.5 µM Cu and 0.8 mM Mg) was determined due to being the highest value. The temperature was increased from 30 °C to 37°C. The activities were detected on 8th and 10th days as 8.67 U L^{-1} and 5.16 U L^{-1}, respectively. Increasing of temperature was resulted by a decrease of PS II activity due to decreased pigment production at 37°C. It may be said the pigments in which were present in PSII complex could be affected with changed temperature. The results, in which effects of temperature changing were researched on cyanobacterium *Synecocystis sp.* PCC6803, were similar (Inoue et al., 2001).

CHAPTER FOUR
CONCLUSION

S. platensis is a planktonic photosynthetic filamentous cyanobacterium. Metals such as Cu and Mg, are essential nutrients and have important physiological and molecular roles in *S. platensis*. Mg is a structural element of chlorophyll molecule and a cofactor for many enzymes and Cu presents in some electron career and also functions in many enzymatic processes such as photosynthesis, pigment production. They play important roles on PS II activity. Therefore, the aim of the study was to investigate the effect of magnesium (0-4.057 mM), copper (0-5 μM) and temperature (37^oC) on the biomass and levels of protein, Chl *a*, phycobiliproteins, carotenoids, MDA, proline, and metal contents of *S. platensis*, as well as PS II activity.

In conclusion, it was demonstrated that different concentrations of Mg or Cu as well as temperature have an important influence on production of biomass, protein, CPC, APC, carotenoids and PSII activity by *S. platensis* in this study. The highest biomass and protein level was obtained at 37^oC while CPC and APC levels were decreased. The highest levels of CPC and APC were obtained at control condition of Cu (0.5 μM) while the highest β-carotene was detected as 2396.73 μg mL^{-1} in absence of Cu leading to stress on the *S. platensis* cells. In addition, increasing Mg concentration was supported carotenoids production and the highest β-carotene was detected as 2354.15 μg mL^{-1} in 1.62 mM Mg conditions, while lack of Mg resulted in a value as 1864.1 μg mL^{-1}. Effect of Mg concentrations on levels of carotenoids produced by *S. platensis* has not been studied until now. However, PSII activities were affected by Mg or Cu concentrations. The inexistence of Mg was more affected to PS II activities compared to lack of Cu condition. Conversely, PS II activities were more decreased when Cu present in excess compared to Mg. Moreover, the temperature of 37^oC also caused to a decreasing on PS II activities. This study sets a good example to production of carotenoids, which are used in several industries such as food, cosmetic, pharmaceutical.

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